Cell Stem Cell
In Translation

Metastatic Cancer Stem Cells: An Opportunity for Improving Cancer Treatment?

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DOI 10.1016/j.stem.2010.05.001

Many human cancers are driven by cancer stem cells (CSCs) whose connection to metastatic spread remains incompletely understood. In this issue of *Cell Stem Cell*, Pang et al. (2010) isolate a subpopulation of human colorectal CSCs that uniquely possesses metastatic potential.

A growing body of evidence has demonstrated that many human tumors are organized as cellular hierarchies initiated and maintained by a subpopulation of self-renewing cancer stem cells (CSCs) (Dalerba et al., 2007; Jordan et al., 2006). These CSCs have been isolated based on their surface immunophenotypes and the ability to serially transplant human cancer in immunodeficient mice, where they give rise to the heterogeneous cell types comprising the original tumor.

Many human cancers, particularly carcinomas, are able to metastasize to secondary sites, and these metastases contribute significantly to patient morbidity and mortality. Cancer stem cells have been identified in both primary and metastatic tumors; however, the relationship between CSCs from these two sources has not been established. Are the same CSCs responsible for both the primary tumor and metastases? Or, in contrast, do distinct CSC subsets exist, one stationary and one migratory, as has been proposed (Brabletz et al., 2005)? Can CSCs capable of metastasis be identified in primary tumors and can the presence of these cells predict the occurrence of metastases?

Data supporting the existence of separable populations of stationary and migratory CSCs come from the investigation of a human pancreatic cancer cell line, in which both the $CD133^+CXCR4^-$ and $CD133^+CXCR4^+$ fractions were capable of sustaining tumor growth, but depletion of the $CD133^+CXCR4^+$ fraction abrogated the formation of metastases (Hermann et al., 2007). While this cell line experiment is provocative, formal demonstration of this model requires experiments with primary human tumors. In the present study, Pang and colleagues demonstrate the existence of a subpopulation of CSCs in primary human colorectal cancer that uniquely possesses metastatic potential (Pang et al., 2010).

The authors examined surface antigen expression on primary colorectal cancers and liver metastases and observed that CD26 was preferentially found on the metastases. Moreover, they determined that all 16 metastases examined contained CD26-positive cells, while such cells were present in only 8 out of 27 primary tumors. Strikingly, 0 out of 19 patients lacking CD26+ cells in the primary tumor developed liver metastases, while 5 of the 8 patients with CD26+ cells ultimately developed liver metastases (the remaining 3 subjects were followed for a much shorter time period). These clinical data strongly link the presence of CD26+ cells in the primary tumor to the development of metastases. But are these CD26+ cells metastatic CSCs?

To investigate this important question, the authors established an orthotopic xenotransplantion assay in which colorectal cancer subpopulations were implanted into the mouse cecal wall. Both $CD26^+$ and $CD26^-$ populations were able to initiate growth of human tumors in the cecum. However, only CD26+ subpopulations established liver metastases in this assay. Consistent with this observation, only $CD26^+$ cells were detected in the portal vein, and unlike $CD26^+$ cells, even direct portal vein injection of $CD26^-$ cells did not result in liver metastases. These results suggest the existence of a subpopulation of metastatic human colorectal CSCs that can be isolated based on its expression of CD26.

These findings have significant clinical implications for the diagnosis and treatment of human colorectal cancer. The identification of subgroups of patients at highest risk of metastasis is an important area of clinical investigation because these patients stand to gain the most from aggressive systemic therapy. The findings by Pang et al. suggest that analysis of CD26 expression in primary tumors could identify early-stage patients who will ultimately develop metastases. Thus, it will be critical to validate this association in large, independent patient cohorts.

Accomplishing this important verification step will require the establishment of routine methods to assess CD26 expression in patient specimens. Pang et al. employ flow cytometry for this purpose, and while this technique is used clinically in certain situations (e.g., hematologic malignancies), it is currently not routinely performed on solid tumor specimens, which are more difficult to process than blood samples. The most straightforward approach would be the use of immunohistochemistry (IHC) because this is routinely performed in clinical laboratories. However, IHC may lack the necessary sensitivity and dynamic range to identify small subsets of $CD26^+$ CSCs in primary tumors. In addition, use of quantitative real-time PCR and other assays on bulk tissues is complicated by the fact that CD26 is expressed by both malignant and normal cells, making it difficult to be certain which cells are contributing to the measured expression. Most likely,

novel technologies and approaches will be required to reliably assess CSC markers, such as CD26, in clinical specimens. Among the leading candidates are devices that can purify circulating tumor cells from peripheral blood and allow subsequent analysis of marker expression (Nagrath et al., 2007; Talasaz et al., 2009). Since CSC responsible for metastasis will most likely be present in the circulation, this represents an intriguing, minimally invasive approach that could overcome the challenges inherent in isolating cells from solid tissues.

Beyond assessment of CD26 expression in primary tumors, it will be important to relate the presence of $CD26^+$ CSCs to our existing molecular understanding of colorectal cancer (Cunningham et al., 2010). Specifically, it will be important to elucidate if $CD26^+$ CSC-containing tumors are enriched among those with the chromosomal instability or microsatellite instability phenotypes or if there is overlap between these tumors and those with derangements in oncogenes such as K-RAS.

Assuming the expression of CD26 by CSCs can be reliably measured in clinical specimens, an important question will be whether the new findings can be used to select patients who will benefit from adjuvant systemic therapies, such as chemotherapy aimed at eliminating microscopic metastases. Currently, the use of such therapies remains controversial in patients with stage II disease (i.e., deeply invading tumors without lymph node metastases) (Cunningham et al., 2010). Since ~75%–80% of stage II patients survive long term even in the absence of adjuvant treatment, it has been difficult to show a clear survival benefit for the addition of chemotherapy (Gill et al., 2004). Extrapolating the findings of Pang et al., it is possible that the subset of stage II patients whose tumors contain $CD26^+$ CSCs are most likely to benefit from systemic treatment. To test this hypothesis, randomized clinical trials could be designed which stratify patients based on the presence of $CD26^+$ CSCs and only administer chemotherapy to the patients most likely to develop metastases. A similar trial design is currently being used to test if loss of heterozygosity at 18q, which correlates with higher risk of metastasis, and microsatellite instability, which correlates with lower risk of metastasis, can be used to select stage II patients for adjuvant treatment (NCT00217737).

The most exciting clinical implication of the current findings is the potential to therapeutically target CD26 for the treatment and/or prevention of metastatic colorectal cancer. Pang et al. show that downregulation of CD26 decreased the migratory and invasive capacities of $CD26^+$ CSCs in vitro. This result suggests that blocking monoclonal antibodies or inhibitory small molecules targeting CD26 could eliminate or inactivate metastatic CSCs. Even if such therapies were not directly cytotoxic to $CD26^+$ CSCs, preventing their ability to metastasize would represent a novel therapeutic approach that could be envisioned as an important component of multimodality cancer therapy.

REFERENCES

Brabletz, T., Jung, A., Spaderna, S., Hlubek, F., and Kirchner, T. (2005). Nat. Rev. Cancer *5*, 744–749.

Cunningham, D., Atkin, W., Lenz, H.J., Lynch, H.T., Minsky, B., Nordlinger, B., and Starling, N. (2010). Lancet *375*, 1030–1047.

Dalerba, P., Cho, R.W., and Clarke, M.F. (2007). Annu. Rev. Med. *58*, 267–284.

Gill, S., Loprinzi, C.L., Sargent, D.J., Thomé, S.D., Alberts, S.R., Haller, D.G., Benedetti, J., Francini, G., Shepherd, L.E., Francois Seitz, J., et al. (2004). J. Clin. Oncol. *22*, 1797–1806.

Hermann, P.C., Huber, S.L., Herrler, T., Aicher, A., Ellwart, J.W., Guba, M., Bruns, C.J., and Heeschen, C. (2007). Cell Stem Cell *1*, 313–323.

Jordan, C.T., Guzman, M.L., and Noble, M. (2006). N. Engl. J. Med. *355*, 1253–1261.

Nagrath, S., Sequist, L.V., Maheswaran, S., Bell, D.W., Irimia, D., Ulkus, L., Smith, M.R., Kwak, E.L., Digumarthy, S., Muzikansky, A., et al. (2007). Nature *450*, 1235–1239.

Pang, R., Law, W.L., Chu, A.C.Y., Poon, J.T., Lam, C.S.C., Chow, A.K.M., Ng, L., Cheung, L.W.H., Lan, X.R., Lan, H.Y., et al. (2010). Cell Stem Cell *6*, this issue, 603–615.

Talasaz, A.H., Powell, A.A., Huber, D.E., Berbee, J.G., Roh, K.H., Yu, W., Xiao, W., Davis, M.M., Pease, R.F., Mindrinos, M.N., et al. (2009). Proc. Natl. Acad. Sci. USA *106*, 3970–3975.